B6

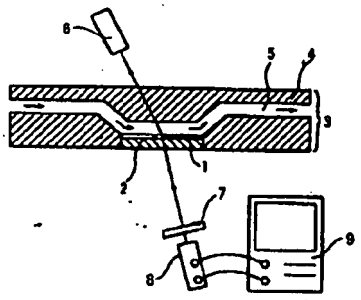
(Abstract)

(Object)

To put forward molecular recognition function membrane which detects the concentration change of specific molecule by converting into change of second harmonic generation intensity, and a sensor using the same.

Construction

A molecular recognition function membrane 1 is formed on a transparent substrate 2, and measurement sample 5 passing through passage 4 in the transparent flow cell 3 is established so as to directly come into contact with the molecular recognition function membrane 1. The light emitted from the light emitting element 6 (fundamental wave) transmits the flow cell 3 and the passage 4 that flows the measurement sample 5, and irradiates the molecular recognition function membrane 1. The light that transmitted the molecular recognition function membrane 1 including the second harmonic wave is filtered for the wavelength other than second harmonic wave using a cut filter 7, and is emitted to optical receptor element 8. The change of second harmonic waves detected is processed by signal processing part 9, and the concentration of the specific sample contained in the measurement sample 5 is calculated.



Patent Claims

Claim 1

A molecular recognition function membrane of the kind wherein a molecular recognition substance having a catalytic function such as enzyme reaction or the like with specific compound, an adsorption function with the said compound or a complex formation function with the said compound is immobilised on a carrier,

characterised in that the aforesaid membrane is the accumulation layer in which monolayers

are accumulated in asymmetric structure, and a compound which generates second harmonic wave is contained in at least one of the plurality of monolayers that construct the said accumulation layer.

Claim 2

A molecular recognition function membrane in accordance with Claim 1, wherein the aforesaid compound which generates the second harmonic waves is a compound having a chromophore placed in between electron donor group and electron accept group.

Claim 3

A molecular recognition function membrane in accordance with Claim 1, wherein the aforesaid molecular recognition function membrane is the accumulation layer formed by accumulating at least one monolayer including the compound having hydrophilic group and hydrophobic group.

Claim 4

A sensor comprising

a molecular recognition function membrane containing a compound which generates second harmonic waves in at least one of the plurality of monolayers which construct the accumulation layer formed by accumulating monolayers in asymmetric structure,

a light emitting element which generates fundamental wave and emits to the aforesaid molecular recognition function membrane,

an optical receptor element which detects the second harmonic waves generated there, and

a signal processing part that processes the signal from the optical receptor element.

Detailed Description of the Invention

(0001)

Sphere of Application in Industry

This invention relates to the following, namely, a molecular recognition function membrane which carries out concentration measurement of organic and inorganic compounds or biological compounds or the like by second harmonic generation, and a sensor using the said membrane.

(0002)

Technology of the Prior Art

The living body has a capability to recognise inorganic and organic substances with high

selectivity. Biosensors have been known which detect a sample in sample solution quickly and simply using functional molecules having such high selectivity, or cells, tissues or even the living body itself as the substance selective functioning part.

(0003)

The function of aforesaid sensor can be regarded by dividing into a part that selectively recognises the specific sample and causes reaction, and a part that senses the change of conductivity, heat generation, luminescence or the like of sample due to the reaction thereof and converts into signal. As biopolymers which recognize the sample and react therewith, proteins species such as enzymes, antibodies, receptors or the like are known, and these are used by dispersing and immobilising in the membrane made of natural polymer or synthetic polymer. On the other hand, as the part that senses the reaction and converts into signal, electrodes such as oxygen electrode, hydrogen peroxide electrode, ion electrode, gas electrode or the like are generally used. Moreover, recently a number sensors and the like using luminescence of the aforesaid sample have been proposed.

(0004)

The electrochemical measurement method using the aforesaid electrode has defects such that (1) electrical and magnetic noises are readily generated during measurement, (2) measurement of trace sample cannot be made due to the use of reference electrode, or the like. Whereas, optical measurement method, which is disclosed in for example Kokai 61-292045, Kokai 63-75542, Kokai 63-271162, has advantages such that (1) high sensitive light detection became possible due to technological innovation of recent years, (2) electrical and magnetic noises are hardly generated during measurement, (3) reference electrode is not necessary, and the like, and furthermore, when an optic fibre is used for the incidence and detection of light, there are characteristics or the like that the reagent exchange in reaction field becomes easy as physical contact between the reaction field and the optic fibre is not necessary, moreover, there is a possibility that plurality of samples can be simultaneously measured using plurality of wavelengths.

(0005)

Meanwhile, organic non-linear optical materials have been studied energetically as material for the aforesaid second harmonic generation, because of the possibility of realizing, in principle, higher nonlinear optical characteristics than inorganic material. In order to realise high second harmonic generation (SHG), molecules with high molecular polarity is needed to be arranged in sequential structure which does not have center of inversion (asymmetric

orientation). Therefore, the study which had been proceeding originally on single crystal material has been carried out recently on accumulation layer having asymmetric orientation structure formed by Langmuir-Blodgett (LB) method (Japan Society of Applied Physics Vol. 60, issue 6 (1991) P586). This LB method has characteristics or the like such that (1) sequence structure which does not have center of inversion (asymmetric orientation) can be easily formed, (2) the thin membrane always having optical axis in the normal direction of substrate is obtained, (3) the thin membrane can be controlled in molecular level, (4) patterning is possible using lithography technique by introducing polymerisable group, (5) refractive index can be controlled by introducing substance such as heavy metal or the like, and moreover, when a molecule with high molecular polarisation is used as film-forming component, there is a great feature that the formed LB membrane itself also shows high polarisation characteristics, and accordingly displaying high second harmonic generation.

(0006)

The aforesaid second harmonic generation is one of characteristics of non-linear optical material, but where a change occurs on the molecular arrangement or molecular structure of LB membrane, the non-linear optical characteristics are also affected. There is an example of applying this principle in which protonation of LB membrane film-forming component was carried out with hydrogen chloride gas and the change in second harmonic during this was analysed (The Electrochemical Society, spring meeting 1991, 2A22).

(0007)

Problems to be Overcome by this Invention

Aforesaid LB method of the prior art can generate second harmonic waves, but how to utilise this as a sensor is still in a research stage, and is not applied in practical use. Even in the study linking the aforesaid change of molecular structure and non-linear optical characteristics, this technique was unsuitable to use as analysis apparatus because the LB membrane constituting molecule receives direct chemical change.

(0008)

Means to Overcome these Problems

In order to overcome the aforesaid problems, this invention forms accumulation layer by introducing a compound having molecular recognition function in a film-forming component of monolayer and a compound that generates second harmonic waves. Accordingly, because this accumulation layer has molecular recognition function, the tertiary structure of molecular arrangement is changed due to adsorption of specific

molecule, reaction catalytic action and the like, and the second harmonic generation function is changed accompanied by this, therefore the concentration of the said specific molecule can be measured by detecting this change. The aforesaid compound having the molecular recognition function and the compound that generates the second harmonic wave may be respectively present in different monolayer film-forming components, and in this case, accumulation layer of asymmetric structure can be easily formed by alternating accumulation of two monolayers.

(0009)

The second harmonic generation property can be intensified by forming the aforesaid compound that generates the second harmonic waves into a compound having chromophore placed in between electron donor group and electron accept group. Moreover, if the aforesaid monolayer includes the compound having hydrophilic group and hydrophobic group, accumulation layer can be readily formed by the aforesaid LB method.

(0010)

Moreover, molecular recognition function membrane in accordance with this invention can measure the concentration of specific molecule by detecting the change of second harmonic waves due to the effect of the specific molecule as described above, and therefore can be used as a sensor. As the structure of a sensor of this invention, generally used Maker-fringe method or wedge method may be followed ("Optical Measurement Handbook" Asakura bookstore IV-1.2.1. p.494), and the sensor comprises a luminescence element which generates fundamental wave and emits to the molecular recognition function membrane, an optical receptor element which detects second harmonic waves generated from the luminescence element and a signal processing part that processes the signal from this optical receptor element, and the concentration can be measured by measuring the second harmonics inherent to the measuring sample generated from the aforesaid molecular recognition function membrane.

(0011)

Action

Because the tertiary structure of molecular arrangement is altered due to adsorption of specific compound, reaction catalytic action or the like, accompanied with this, the second harmonic generation function is also changed, thereby the molecular recognition function membrane of this invention can measure the concentration of specific compound by detecting this change.

(0012)

Examples

Below, Examples of this invention are described. Figure 1 is a schematic diagram to illustrate a sensor on the basis of this invention. A molecular recognition function membrane 1 is formed on a transparent substrate 2, and a measurement sample 5 passing through the passage 4 in the transparent flow cell 3 is established so as to directly come into contact with the molecular recognition function membrane 1. The light emitted from the light emitting element 6 (fundamental wave) transmits the flow cell 3 and the passage 4 flowing the measurement sample 5 and irradiates the molecular recognition function membrane 1. The light that transmitted the molecular recognition function membrane 1 including the second harmonic wave is filtered for the wavelength other than second harmonic wave using a cut filter 7, and is emitted to optical receptor element 8. The change of second harmonic waves detected in this way is processed in signal processing part 9, and the concentration of the specific sample contained in the measurement sample 5 is calculated.

(0013)

Figure 2 to Figure 5 show molecular recognition function membrane 1 of this invention and constituting molecules thereof. The molecular recognition function membrane 1 of Figure 2(a) is a membrane wherein monolayer A formed from molecule 10 and molecule 11 and monolayer B formed from molecule 10 and molecule 12 are accumulated in a form of hetero Y type structure described later. These monolayers can be readily made into the accumulation layer by LB method. Only two layers are illustrated in the Figures, but the form s not limited to two layers in practice.

(0014)

Molecules 10 and 11 have structures shown in Figure 3. In other words, the molecule 10 comprises hydrophobic group 13 and hydrophilic group 14 as shown in Figure 3 (a), and moreover the molecule 11 comprises hydrophobic group 15, hydrophilic group 16 and chromophore 17. The hydrophobic group 15 in the molecule 11 also acts as electron donor group and the hydrophilic group 16 also acts as electron accept group, however, of course the hydrophobic group and the electron donor group, the hydrophilic group and the electron accept group may be separate group. Moreover, the hydrophobic group 13 and the hydrophilic group 14 to be used in the molecule 10 may be any kind of well known groups, however, on consideration of the affinity to the molecule 11, respectively the same one or

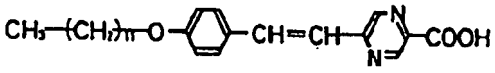
the same kind of hydrophobic group 15 also acting as electron donor group and the hydrophilic group 16 also acting as electron accept group are preferably used. As hydrophobic group 15 also acting as electron donor group, alkyl group or aryl group is preferred, and as the hydrophilic group 16 also acting as electron accept group, -COOM group, $-SO_3M$ group and the like are preferred (M denotes a hydrogen atom or alkaline earth metal atom or alkali metal atom).

(0015)

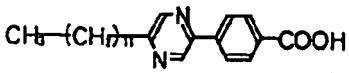
Moreover, as polar chromophore 17, well known species such as nitro group, azo group, conjugated diene and the like can be used, and the said chromophore 17 is preferably placed in between the electron donor group and the electron accept group. From the above, the compounds such as arachic acid, barium stearate, stearyl alcohol, stearyl mercaptan, stearylamine and the like are nominated as the molecule 10 and moreover, as the molecule 11, the compounds shown for example in Formula 1 to Formula 5 may be nominated. Moreover, the molecule 12 is a compound having function to distinguish the specific molecule and to receive action thereof. As the molecule 12, for example as the compound that adsorbs specific molecule, Crown ether having function to adsorb only the ion having specific contour may be proposed. Enzymes that only catalyses reaction of specific substrate may be proposed, too.

(0016)

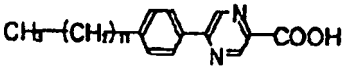
Compound 1



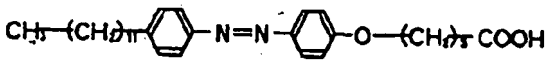
Compound 2



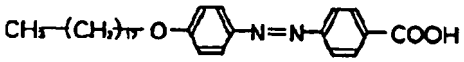
Compound 3



Compound 4



Compound 5



(0017)

Figure 4 is a schematic diagram illustrating a type of accumulation of monolayer by LB method. In the Figure, (b) has a symmetric type structure (Y type structure) in which hydrophilic groups form contact to each other (moreover, although not shown in Figure, hydrophobic groups also form contact to each other), and is a stable membrane. However, because this accumulation layer is symmetric, the membrane as a whole is not polarized, and accordingly, the second harmonic generation capability is inferior. On the other hand, (a) (X type structure) and (c) (Z type structure) are asymmetric structures in which all the molecules in accumulated monolayer are facing one direction. Accordingly, the whole membrane is polarized due to the effect of electron donor group and electron accept group in the molecule, and the second harmonic generation can be carried out efficiently. However, unlike the symmetric structure of (b), because the hydrophilic group and the hydrophobic group form a contact, it is a rather unstable membrane. On the other hand, as for the structure of (d), two kinds of monolayers are accumulated in alternation, and by looking at as membrane unit of each species, it has asymmetric structure in which all the molecules are facing one direction (hetero Y type structure). Because this hetero Y type structure is the structure in which hydrophilic groups form contact to each other (moreover, although not shown in Figure, hydrophobic groups also form contact to each other), and is a stable membrane, and it is the most desirable form as the asymmetric structure in accordance with this invention.

(0018)

When accumulation layer of the aforesaid hetero Y type structure is to be formed, for example a case of forming the layer as in Figure 2 (a) is described. Monolayer A comprises from the molecule 10 having hydrophobic group and hydrophilic group and molecule 11 having electron donor group, electron accept group and chromophore. Accordingly, the monolayer A is a membrane having second harmonic generation function. Moreover, monolayer B comprises the molecule 10 having hydrophobic group and hydrophilic group

and molecule 12 having function to distinguish specific molecule and to receive action thereof. In other words, the monolayer B is a molecular recognition function membrane. Accumulation layer is formed by further accumulating monolayer A, monolayer B, monolayer A, monolayer B, ... on this monolayer B, however, all the hydrophilic groups (electron accepter groups) are facing upward in the molecules in plurality of monolayer A, and all the hydrophilic groups are facing downward in the molecules in plurality of monolayer B (when molecule 12 is an enzyme, because enzymes are often hydrophilic, it align on the side of hydrophilic group of molecule 10, and moreover, when the molecule 12 is hydrophobic, it can be aligned on the side of hydrophilic group in the same way by addition of alkyl group or the like). Accordingly, looking as membrane as a whole, it becomes membrane of asymmetric structure, and the monolayer A becomes a form as if plane batteries are connected in series, polarization occurs into – at the membrane upper part and + at the membrane lower part, and second harmonic generation can be carried out efficiently.

(0019)

When the aforesaid membrane is used as sensor, although the mechanism thereof is not clear, the compound having molecular recognition function in the molecule 12 generates deformation such as expansion, distortion or the like due to the action of specific molecule, and it is thought that this deformation is communicated to chromophore-containing molecule 11 of monolayer A, and affects directional property or the like of molecule 11. In any case, the change occurs in the second harmonic generation function inherent to the action of specific molecule, and besides, this change has a correlation to the concentration of specific molecule, therefore the concentration of specific molecule can be measured using this.

(0020)

Figure 2(b) is an example of one kind of monolayer having both the second harmonic generation function and the molecular recognition function. When molecule 12 having molecular recognition function is hydrophilic, it can be immobilised in the monolayer by molecule 10 having hydrophilic group and hydrophobic group as seen in this Figure. When forming accumulation layer by LB method, there is a method to accumulate this one kind of monolayer, but formation is easier and stability is better when a structure of said hetero Y type is formed using for example monolayer formed from the molecule having hydrophilic group and hydrophobic group such as molecule 10.

(0021)

Figure 2(c) is an example of one kind of membrane having both the second harmonic generation function and the molecular recognition function. Because molecule 12 having molecular recognition function is hydrophobic, this membrane is in a form of being carried over to the hydrophobic group side of membrane when the monolayer is formed on the water surface. This membrane can be readily formed into the accumulation layer using LB method, too. Moreover, in this case, in the same way as in the example of the aforesaid (b), formation is easier and stability is better when a structure of said hetero Y type is formed using for example monolayer formed from the molecule having hydrophilic group and hydrophobic group such as molecule 10.

(0022)

The aforesaid accumulation layer using LB method wherein two kinds of membranes are accumulated alternatively can be formed using for example two-trough system Langmuir trough 18 as shown in Figure 5. Trough I and trough II for forming monolayer are established in two-trough system Langmuir trough 18. The trough I and trough II are filled with water, and to each water surface is added dropwise each composition of monolayer A and B of for example Figure 2(a) dissolved in organic solvent. This organic solvent is volatilised, and only the component of each monolayer is developed as gaseous membrane. Each monolayer formed in this way is compressed with the barrier which is not illustrated, and condensed film is made by compressing at a desired surface pressure.

(0023)

A part where the membrane of transparent substrate 2 is formed is immersed in trough I as shown in Figure in a state in which the monolayers have been formed in trough I and trough II as described above. During this procedure, barrier is moved so that the surface pressure does not change even when the monolayer A is moved to the transparent substrate 2. Any substrate having transparency which is not deteriorated by sample or solvent may be used as transparent substrate 2 in this invention, and for example substrate formed from inorganic material such as glass plate or the like and organic polymer such as acryl plate or the like may be proposed. Next, transparent substrate 2 is moved to trough II by passing through flexible gate 19a, 19b while being immersed state. The transparent substrate 2 moved to the trough II is shown with imaginary line. Moreover, the trough III established between flexible gate 19a and 19b is an intermediate tank provided in order to prevent mixing of two kinds of monolayers. The monolayer B is accumulated on monolayer A by pulling up the membrane forming part of the transparent substrate 2 transferred to the trough II. Then, the

transparent substrate 2 is returned to the trough I by passing through flexible gate 19b, 19a while in the elevated state, and then the membrane forming part is immersed. Accumulation layer can be obtained by repeating the above procedures.

(0024)

Embodying Examples of this invention are described below.

Example 1

As transparent substrate 2, transparent glass plate of refractive index nd= 1.523 was washed and used. Using two-trough system Langmuir trough 18 showed in Figure 5, a chloroform solution of compound of Formula 1 (the compound including chromophore belonging to molecule 10 of Figure 3) and arachidic acid (the compound having hydrophobic group and hydrophilic group belonging to molecule 10 of Figure 3, CH3(CH2)18COOH) was added dropwise in trough I to form monolayer A, and it was compressed at a surface pressure of 25 mN/m. On the other hand, a chloroform solution of an enzyme, glucose oxidase (the compound having molecular recognition function belonging to molecule 12 of Figure 2) and enzyme immobilising agent DPPE (the compound having hydrophobic group and hydrophilic group belonging to molecule 10 of Figure 3 Dipalmitoyl phosphatidyl ethanolamine, Sigma Corp.) was added dropwise in trough II to form monolayer B, and it was compressed at a surface pressure of 8 mN/m. As for these enzyme and enzyme immobilising agent, well known various kinds can be used other than above-mentioned species.

(0025)

Thereafter, according to the aforesaid accumulation layer forming procedure, hetero Y type structured LB membrane was formed in which monolayers A and B were accumulated alternatively in total of 81 layers on the transparent substrate 2. Thereafter, one side of the formed membrane was eliminated by exposing only one side of transparent substrate 2 to chloroform. Using membrane formed on this transparent substrate 2, firstly, the second harmonic intensity was measured in untreated state, then the measurement was carried out after exposing the membrane surface to pure water for five minutes, and finally the measurement was carried out after exposing the membrane surface to glucose aqueous solution of 100 mg/ml concentration for five minutes. As a result, the untreated second harmonic waves and the second harmonic wave treated with pure water showed almost same intensity, but the second harmonic waves contacted with glucose aqueous solution showed apparent lowering of the intensity. Moreover, the measurement of second harmonic

intensity was carried out as follows. The transparent substrate 2 on which the aforesaid membrane was formed was irradiated from the membrane side using fundamental wave (λ = 1.064 μm) of YAG laser, and detection was carried out by eliminating the fundamental wave in the transmitted beam using infrared radiation cut filter.

(0026)

Example 2

Firstly, two pieces of transparent glass plates with refractive index nd= 1.523 were prepared as transparent substrate 2, and Cr membrane (2 nm) was formed beforehand by vacuum deposition method on one side of one of them. Using two-trough system Langmuir trough 10 showed in Figure 5, a chloroform solution of compound of Formula 2 (the compound including chromophore belonging to molecule 11 of Figure 3), crown ether (the compound having molecular recognition function belonging to molecule 12 of Figure 2, Bis(12Crown4)) and arachidic acid (the compound having hydrophobic group and hydrophilic group belonging to molecule 10 of Figure 3, CH3(CH2)18COOH) was added dropwise in trough I to form monolayer A, and it was compressed at a surface pressure of 23 mN/m. On the other hand, a chloroform solution of the same composition used in trough I except using the compound of Formula 3 instead of the compound of Formula 2was added dropwise in trough II, and monolayer B was formed. The surface pressure was adjusted to 23 mN/m in the same way. Various well known species can be used as the aforesaid crown ether other than above-mentioned, but the bicyclic system of this example is preferable, because when a specific ion is adsorbed, torsion is generated in the structure of crown ether, and physical change of membrane structure becomes markedly, and therefore the change of secondary harmonic intensity is also large.

(0027)

Thereafter, firstly, the glass transparent substrate 2 on which Cr membrane had been formed on one side beforehand was immersed in trough II and lifted in trough I, and this procedure was repeated ten times, and 20 layers of hetero Y type membrane was formed. Thereafter, the side opposite to the side on which Cr membrane was formed was exposed to chloroform, and the membrane formed on this side was eliminated. On the other hand, the glass transparent substrate 2 on which Cr membrane had not been formed was firstly immersed in trough I and then lifted in trough II, and this procedure was repeated ten times, and 19 layers of hetero Y type membranes were formed. Thereafter, one side of the formed membrane was eliminated by exposing one side to chloroform. Next, the sides of these two substrates from which membranes had been eliminated were laminated to each other, and

the substrate provided with membrane on both sides on the basis of this invention was completed.

(0028)

Thereafter, using the same YAG laser as in Example 1, generated second harmonic intensity was measured while rotating the aforesaid substrate (in other words, while changing the angle of incidence), and fringe pattern shown in Figure 6 was obtained. The reason why such pattern was generated is that the second harmonic waves generated in each membrane interfere with each other, and the reason why it changed according to the angle of incidence is due to the change of path length. Therefore, the aforesaid substrate was fixed at the angle wherein the second harmonic became the minimum value, and it was exposed to pure water for five minutes, and thereafter the second harmonic intensity was measured, as a result, no change was observed. Thereafter, the measurement was carried out after exposing to sodium chloride aqueous solution in concentrations of 2 mol/l and 10 mol/l for five minutes, as a result, marked increase of the second harmonic intensity in correlation with concentration change was observed.

(0029)

Comparative Example 1

Accumulation layer was formed on glass transparent substrate 2 in the same way as in Example 1, except that glucose oxidase was not added to the composition for formation of monolayer B, and the second harmonic intensity was measured. As a result, the change in intensity did not occur even when the membrane surface was treated with glucose aqueous solution of 100 mg/ml concentration.

(0030)

Comparative Example 2

Accumulation layer was formed on glass transparent substrate 2 in the same way as in Example 1, except that the compound of Formula 1 was not added to the composition for formation of monolayer A, and the second harmonic intensity was measured. As a result, the second harmonic wave could not be detected in any case of untreated, pure water treated and treatment with glucose aqueous solution 100 mg/ml concentration.

(0031)

Advantages Afforded by this Invention

As described above, in accordance with this invention, the concentration of specific

molecule can be readily detected, because various actions such as adsorption function of specific compound, complex forming function with this compound or catalytic action such as enzyme reaction, immunoreaction or the like of the said compound can be detected by converting to the change in the second harmonic generation intensity.

Brief Description of the Figures

Figure 1

A schematic diagram showing a sensor on the basis of this invention.

Figure 2

A schematic figure showing a part of molecular recognition function membrane in accordance with this invention.

Figure 3

A schematic figure showing a part of component molecule constituting the molecular recognition function membrane in accordance with this invention.

Figure 4

A schematic figure showing accumulation forms by LB method of molecular recognition function membrane in accordance with this invention.

Figure 5

A schematic diagram of two-trough system Langmuir trough.

Figure 6

A correlation diagram of the angle of incidence of the fundamental wave and the generated second harmonic intensity.

Key to Symbols

1... molecular recognition function membrane, 2... transparent substrate, 3... flow cell, 4... passage, 5... measurement sample, 6... light emitting element, 7... cut filter, 8... optical receptor element, 9... signal processing part, 10,11,12... molecules, 13... hydrophobic group, 14... hydrophobic group, ... hydrophobic group also acting as electron donor group, 16... hydrophilic group, ... acting as electron accept group, 17... chromophore, 18... two-trough system Langmuir trough, 19... flexible gate.

Figure 1

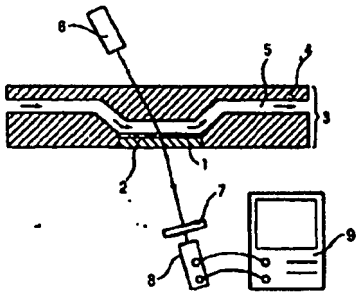


Figure 2

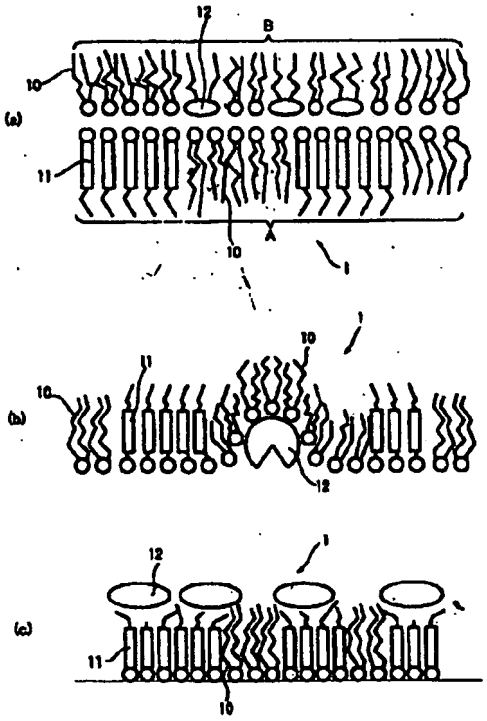


Figure 3

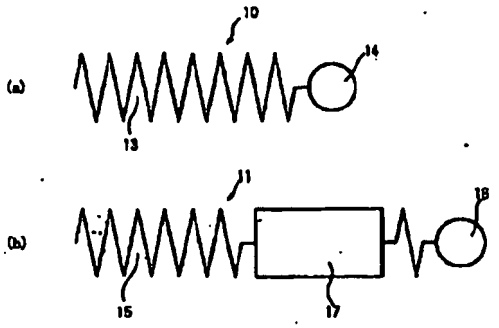


Figure 4

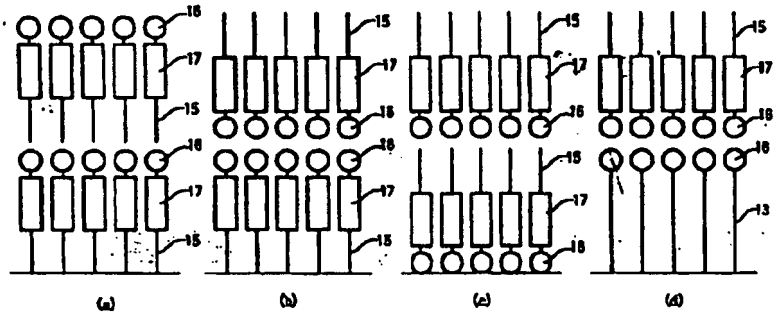


Figure 5

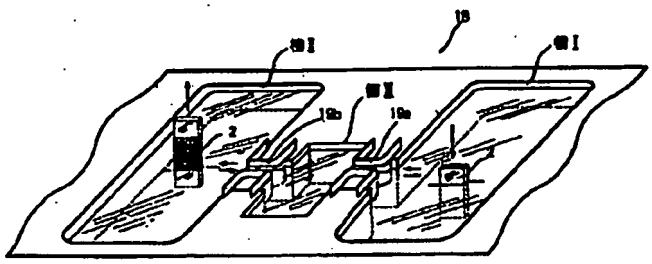
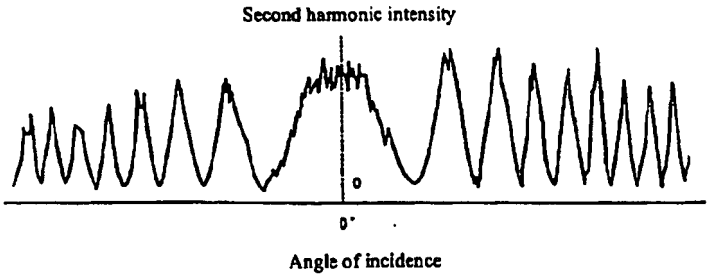


Figure 6



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